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MYCOBACTERIUM VACCAE FOR TREATMENT OF LONG TERM AUTOIMMUNE CONDITIONS

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(57) Claim

1. A method for the prophylaxis or treatment of vascular diseases or complications which are immunologically mediated including those associated with tuberculosis or diabetes which comprises administering to the patient suffering from such a condition an effective amount of antigenic and/or immunoregulatory material derived from *Mycobacterium vaccae*.

7. A product comprising antigenic and/or immunoregulatory material derived from *Mycobacterium vaccae* when used in the prophylaxis or treatment of vascular diseases or complications which are immunologically mediated including those associated with tuberculosis or diabetes.

9. A pharmaceutical agent when used in the prophylaxis or treatment of vascular diseases or complications which are immunologically mediated including those associated with tuberculosis or diabetes which agent comprises antigenic and/or immunoregulatory material derived from *Mycobacterium vaccae*.

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ORIGINAL

COMPLETE SPECIFICATION

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Complete Specification for the invention entitled:

Mycobacterium vaccae for treatment of long term autoimmune conditions

The following statement is a full description of this invention, including the best method of performing it known to us:

MYCOBACTERIUM VACCAE FOR TREATMENT OF LONG TERM AUTOIMMUNE CONDITIONS

This invention relates to the treatment of mental diseases associated with an autoimmune reaction initiated by an infection, and of the auto-immunologically mediated consequences (other than uveitis) of chronic infections.

British Specification No. 2156673 describes immunotherapeutic agents comprising killed cells of Mycobacterium vaccae. These agents are useful in the immunotherapy of mycobacterial disease, especially tuberculosis and leprosy. It is stated that use of this immunotherapeutic agent facilitates the removal of the persisting bacilli responsible for tuberculosis or leprosy which, as is well known, it is difficult to remove by chemotherapy alone. It is suggested in the specification that the immunotherapeutic agent is believed to act by presenting the "protective" common mycobacterial antigens to advantage and by containing immune suppressor determinants which are active in regulating disadvantageous immune mechanisms. As a consequence, "persister" bacilli are recognised by the immune system by their content of common mycobacterial antigens and effective immune mechanisms are directed against them, in the absence of the tissue necrotic form of immunity usually present in mycobacterial disease.

International Patent Specification PCT/GB85/00183 describes compositions for the alleviation of the symptoms of, and for the treatment or diagnosis of, arthritic diseases which comprise as active ingredient the whole organism of M. vaccae. It is stated that the preparations of M. vaccae are useful for the treatment of various autoimmune diseases and especially

arthritic conditions including rheumatoid arthritis, ankylosing spondylitis or Reiter's syndrome.

International Patent Specification PCT/GB90/01318 discloses that compositions which comprise M. vaccae as active ingredient are useful in the treatment of other pathological conditions in which the patient shows an abnormally high proportion of agalactosyl IgG and also in the treatment of chronic inflammatory disorders caused or accompanied by an abnormally high release by macrophages of interleukin-6 and/or tumour necrosis factor. The specification refers in particular to the treatment of Crohn's disease, reactive arthritis, primary biliary cirrhosis, sarcoidosis, ulcerative colitis, psoriasis, systemic lupus erythematosus (especially when accompanied by Sjogren's syndrome), multiple sclerosis, Guillain-Barre syndrome, primary diabetes mellitus, and some aspects of graft rejection.

International Patent Specification PCT/GB90/01169 discloses antigenic and/or immunoregulatory material derived from M. Vaccae for use in delaying or preventing the onset of the AIDS syndrome.

Additionally, our unpublished International Patent Specification PCT/GB91/01970 discloses the use of M. vaccae in the treatment of uveitis, an immunologically mediated late consequence of leprosy, which causes blindness.

The present invention is based on the surprising observation that M. vaccae is also effective against a number of other conditions which may involve infections such as bacterial or protozoan infections and in particular mycobacterial infections.

It has been suggested that mental diseases such as schizophrenia and manic depression are associated with an autoimmune reaction resulting from past or on-going cryptic infection. Evidence for this is provided by abnormal B and T lymphocyte function such as increased B cells, decreased T cells and altered suppressor cell levels which have been observed in schizophrenia (see for example P. Sirota, Israel J. Med. Sci, 1990, 26, 694-697; J.G. Knight, Meth and Find Exptl Clin; Pharmacol., 1984, 64, 395-403; H.H. Fudenberg et al, Biomedicine & Pharmacotherapy, 1984, 38, 285-290; F. Villemain et al, Annal. N.Y. Acad. Sci., 1987, 496, 669-675; and Ganguli et al, Annal. N.Y. Acad. Sci., 1987, 496, 676-685). Evidence is also provided by the observation that schizophrenia and rheumatoid arthritis almost never occur in the same patient (T.D. Spector, J. Silman, Brit. J.. Rheumatology, 1987, 26, 307-310). We believe that schizophrenia and rheumatoid arthritis may be caused by genetically determined, mutually exclusive, reactions to cryptic infection and probably mycobacterial infection. In a limited trial we have found that three out of three patients suffering from schizophrenia have shown a dramatic improvement when treated with M. vaccae. We therefore believe M. vaccae may be useful in treating mental diseases associated with an autoimmune reaction.

We have also found that M. vaccae is effective in the treatment of the immunologically mediated consequences of chronic infections.

Chaga's disease (South American Trypanosomiasis) is an example of a disease associated with a protozoan infection a late consequence of which is myocarditis which normally leads

to the death of a patient. Treatment with M. vaccae may reduce the incidence of myocarditis.

Another example of such disease is Takayasu's arteritis which is associated with tuberculosis which is caused by mycobacterial infection. It is believed that this and other vascular diseases including obstructive vascular diseases, which may be immunologically mediated, may be prevented and treated with M. vaccae.

It is also believed that M. vaccae may be effective in the prophylaxis and treatment of vascular complications associated with diabetes, which may be immunologically mediated.

The present invention accordingly provides a method for the prophylaxis or treatment of vascular diseases or vascular complications which are immunologically mediated, such as those associated with tuberculosis or diabetes, which method comprises administering to the patient suffering from such a condition an effective amount of therapeutic composition comprising antigenic and/or immunoregulatory material derived from Mycobacterium vaccae.

The invention further provides the use of antigenic and/or immunoregulatory material derived from M. vaccae in the manufacture of a therapeutic agent for the prophylaxis or treatment of vascular diseases or vascular complications which may be immunologically mediated, such as those associated with tuberculosis or diabetes.

The therapeutic agent of the invention conveniently, and therefore preferably, comprises dead cells of M. vaccae, most preferably cells which have been killed by autoclaving or by irradiation. The therapeutic agent normally comprises more than 10^8 microorganisms per ml of diluent, and preferably from 10^8 to 10^{11} killed M.

vaccae microorganisms per ml of diluent.

The diluent may be pyrogen-free saline for injection alone, or a borate buffer of pH 8.0. The diluent should be sterile. A suitable borate buffer is:

$Na_2B_4.10H_2O$	3.63 g
H_3BO_3	5.25 g
NaCl	6.19 g
Tween 80	0.0005%
Distilled Water	to 1 litre

The preferred strain of M. vaccae is one denoted R877R isolated from mud samples from the Lango district of Central Uganda (J.L. Stanford and R.C. Paul, Ann. Soc. Belge Med, Trop. 1973, 53 141-389). The strain is a stable rough variant and belongs to the aurum sub-species. It can be identified as belonging to M. vaccae by biochemical and antigenic criteria (R. Bonicke, S.E. Juhasz., Zentr albl. Bakteriol. Parasitenkd.

Infection skr. Hyg. Abt. 1, Orig., 1964, 192, 133. The strain denoted R877R has been deposited under the Budapest Convention at the National Collection of Type Cultures (NCTC) Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, United Kingdom on February 13th, 1984 under the number NCTC 11659.

For the preparation of the therapeutic agent, the microorganism M. vaccae may be grown on a suitable solid medium. A modified Sauton's liquid medium is preferred (S.V. Boyden and E. Sorkin., J. Immuno, 1955 75, 15) solidified with agar. Preferably the solid medium contains 1.3% agar. The medium inoculated with the microorganisms is incubated aerobically to enable growth of the microorganisms to take place, generally at 32°C for 28 days. The organisms are harvested, then weighed and suspended in a diluent. The diluent may be unbuffered saline but is preferably borate-buffered and contains a surfactant such as Tween 80 as described above. The suspension is diluted to give 100mg of microorganism/ml. For further dilution, borate buffered saline is preferably used so that the suspension contains 10 mg wet weight of microorganisms/ml in multidose vials. Although the microorganisms in the vials may be killed using irradiation e.g. from ^{60} Cobalt at a dose of 2.5 megarads, or by any other means, for example chemically, it is preferred to kill the microorganisms by autoclaving, for example at 15 psi (103.5 kPa) for 15 minutes (115°-125°C). It has been discovered that autoclaving yields a more effective preparation than irradiation.

The therapeutic agent is in general administered by

injection in a volume in the range 0.1-0.2 ml, preferably 0.1ml given intradermally. A single dosage will generally contain from 10^7 to 10^{10} killed M. vaccae microorganisms. It is preferred to administer to patients a single dose containing 10^8 to 10^9 killed M. vaccae. A single dose may be administered or the dose may be repeated depending on the condition of the patient.

Although the therapeutic agent will generally be administered by intradermal injection, other routes, e.g. oral administration, can also be used.

It may be advantageous and is within the scope of the invention to use more than one strain of M. vaccae, and/or to include in the antigenic on immunoregulatory material other closely related mycobacterial species, such as M. nonchromogenicum or M. chitae. Tuberculin may also be included.

The therapeutic agent can contain further ingredients such as adjuvants, preservatives, stabilisers etc. It may be supplied in sterile injectable liquid form or in sterile freeze-dried form which is reconstituted prior to use.

M. vaccae may be used as such or an extract or fractioned portion of the organism to manufacture the therapeutic agents according to the invention.

The following Example illustrates the invention.

EXAMPLE

M. vaccae NCTC 11659 is grown on a solid medium comprising modified Sauton's medium solidified with 1.3% agar. The medium is inoculated with the microorganism and incubated for 28 days at 32°C to enable growth and maturation of the

microorganism to take place. The microorganisms are then harvested by gently scraping the surface of the agar and weighed (without drying) and suspended in M/15 borate buffered saline at pH8 to give 10 mg of microorganisms/ml of saline. The suspension is dispensed into 5 ml vials, and then autoclaved for 15 minutes at 15 psi (103.5 kPa) to kill the microorganisms. After cooling, the therapeutic agent thus produced is stored at 4°C before use. A single dose consists of 0.1 ml of the suspension, which should be shaken vigorously immediately before use, containing 1 mg wet weight of *M. vaccae*. The dose is given by intradermal injection normally over the left deltoid muscle.

EXAMPLE 2

The following Example demonstrates the effect of treatment with *M. vaccae* (MV) on the vascular aspects of leprosy. In particular, the bloodflow at the fingertips and the fingertips skin temperature is measured. It is clear from the study that leprosy patients receiving *M. vaccae* immunotherapy were improved in the continuing vascular aspects of the disease.

1. The work consisted of laser Doppler flowmetry measurements of blood flow and skin temperature measurements at the finger-pulp skin of leprosy patients. These parameters were measured in order to study the effect of MV immunotherapy on the impairment of peripheral circulation in leprosy patients.

2. The study was a blinded, randomised, placebo-controlled

study. Patients were randomised into two groups: one group had received *Mycobacterium vaccae* immunotherapy (a single intradermal injection of 0.1 ml (10^9 killed *M. vaccae*) and the other group saline (placebo) injections. Both sets of patients had been treated eighteen months previously. The patients were allocated on a random basis (blind) and the data were gathered and analysed personally.

3. Leprosy patients often show a progressive deterioration in nerve function in the years following infection, with serious consequences such as ulcer formation of the lower limbs and progressive clawing of fingers. Microcirculatory impairment complicates prognosis, especially in patients of long-standing, resulting in disproportionately cold fingers at high ambient temperatures.

4. Methods

At their first visit, 103 patient volunteers, all residents of Baba Baghi Leprosy Hospital, Tabriz, Iran, under the age of 65, were skin tested with 4 common mycobacterial antigens and the results recorded. These patients were then randomly assigned to two groups. The first group (immunotherapy group, IT) were given one intradermal injection of 0.1 ml 10^9 killed *M. vaccae* and the second group (saline group, SAL) were given one 0.1 ml intradermal injection of saline.

5. At the second visit, 18 months after the first, 92 of these patients were still resident at the leprosy hospital and 84 (44 SAL, 40 IT) underwent repeat testing with the 4 common antigens. In addition, all 92 patients (45 SAL, 47 IT) had laser Doppler measurements of fingertip blood flow, vasomotor reflexes and finger sensation. All results were recorded by an operator who was unaware of the subject's IT or SAL status at the time of measurement. Since the measurements of blood flow and sensation could be done only at the second visit, 20 healthy subjects were chosen as controls; 10 were the adult offspring of treated leprosy patients and were residents at Baba Baghi Leprosy Hospital and 10 were apparently healthy members of the staff.

6. Measurement of blood flow by laser Doppler flowmetry (LDF)

The laser Doppler flowmeter (Perimed PF2, Perimed, Sweden) measures the movement of erythrocytes in the most superficial 1 mm of skin from changes in the frequency of coherent light reflected out of the tissue. The instrument is internally standardised and gives an integrated measurement of microvascular blood flow (LDFlux, expressed in Volts) which is related to the product of the number of moving erythrocytes and their mean velocity at the measurement site.

7. The fibre-optic tip was attached to the pulp of the distal phalanx by a probe holder fixed with double-sided

adhesive tape ensuring optimal alignment between sensor head and skin surface. The output signal was recorded continuously during the period of observation on a chart recorder (SE120, BBC Goetz, Metrawatt, Austria) calibrated to a full scale deflection of 10V. The pulp of the finger was selected for investigation because its abundant arteriovenous anastomoses are under strict autonomic control.

8. Measurement of fingertip skin temperature.

A platinum skin thermistor attached to an LCD output device was used to measure skin surface temperature. The probe (Model 4098, 9 mm diameter, Yellow Springs Instrument Co. Inc., Yellow Springs, Ohio, USA) was held in close contact with the skin with a single strip of Millipore adhesive tape. A stable temperature was generally achieved after 5 min contact with the skin of the pulp of the fingertip. The same sensor was used to measure atmospheric temperature near the subject during the experimental procedure.

9. Experimental protocol.

Each subject was seated comfortably with the forearm and hand resting on a table at heart level at an ambient temperature of 25-29°C. This temperature was maintained by a large paraffin stove situated in the corner of the experimentation room. Ambient temperature was measured by a thermistor, described above, situated within 25 mm

of the subject at the same level as the hands. These conditions would be expected to induce near maximal peripheral vasodilation and a stable blood flow through the fingertips in healthy subjects. Each subject was allowed to equilibrate under these conditions for at least 15 min before measurements were started. All four fingers - index (2), middle(3), ring(4) and small (5) were studied on both the right and left hand under investigation. Skin temperature was first recorded, followed by LDFlux.

10. Statistical Analysis.

Differences between patient groups and control subjects for each measured parameter were assessed using Two-way ANOVA taking intra-individual differences between fingers into account.

11. Results.

The table which follows shows the effect of *M. vaccae* immunotherapy on LDFlux and fingertip skin temperature. The 95% confidence intervals for 20 healthy control subjects were 3.1-10.0 Volts for LDFlux (mean 6.6) and 31.0-35.0°C for skin temperature (mean 33.1°C). Consequently leprosy patient fingers with fingertip skin temperature measurements and LDFlux measurements of less than 31.0°C and 3.1 Volts respectively were considered impaired for that parameter.

12. LDFlux measurements and skin temperature were greater ($P<0.05$) in the IT group than the SAL and were lower in both groups ($P<0.05$) than in control subjects. In the SAL group 45.7% of all fingers had impaired LDFlux values (<3.1V) and 42.4% had impaired skin temperatures (<31.0°C) whereas these values were 33.1 and 31.4% respectively for the IT group. In both groups the fourth and fifth fingers of each hand had lower LDFlux values than their respective counterparts in the healthy control group ($P<0.05$).

13. Comparing group results by disease classification, lepromatous patients undergoing IT had significantly higher LDFlux values than those undergoing SAL (Mean values 4.59 vs 3.91, representing 27% vs 42% impaired fingers respectively in each group). Tuberculoid patients showed a similar difference between IT and SAL groups but this did not reach a level of significance (mean values 4.06 vs 3.5). SAL subjects were found to have impaired temperature sensation compared with IT subjects.

14. The effect of *M.vaccae* immunotherapy (IT) or saline (SAL) administration on measurements of fingertip skin blood flow (LDFlux) and temperature.

	Control	SAL	IT
n (subjects)	20	45	47
n (fingers)	160	356	369
Room Temperature (°C)	25.2(0.5)	25.1(1.2)	25.3(1.0)
Age (years)	31.5(13.4)@	45.9(9.3)	46.2(12.5)
Residence at leprosy hospital (years)		22.1(8.9)	22.7(8.7)
LDFlux (V)	6.6(1.7)	3.7(2.8)*@	4.4(2.7)*
Fingers in group with impaired LDFlux (%)	0.6	45.7*@	33.1*
Fingers in group with LDFlux <IV(%)	0	24.6*	12.5*
Fingertip skin temperature (°C)	33.1(1.0)	30.4(3.4)*@	31.0(3.3)*
Fingers in group with impaired fingertip skin temperature (%)	3.8	42.4*@	31.4*

@ < immunotherapy group (P<0.05)

* < Control (P<0.05).

15. The results obtained demonstrated that those patients who had received *Mycobacterium vaccae* immunotherapy eighteen months previously had significantly higher mean laser Doppler flowmetry blood flows and higher mean skin temperatures in the finger-pulp than the corresponding control group.

EXAMPLE 3

This Example relates to treatment of a contact of a leprosy patient who was found to have positive serological tests for *Trypanosoma cruzi*. Following treatment with *M. vaccae*, the patient was able to confer recognition of common mycobacterial antigens. *T. cruzi* infection has been

associated with a lack of response to such antigens.

1. A household contact of a leprosy patient attending the Hospital of Reconquista-Province of Santa Fe, was found to have positive serological tests for *Trypanosoma cruzi* (indirect immunofluorescence and indirect haemagglutination). This person did not respond to common mycobacterial antigens on skin testing with 4 intradermal injections of soluble mycobacterial reagents: left forearm: tuberculin (0.2μgr/0.1ml)=10mm; leprosin A (1μgr/0.1ml)=4mm; right forearm: scrofulin (0.2μgr/0.1ml)=0mm, vaccin(0.2gr/0.1ml)=6mm.

2. A single injection of *Mycobacterium vaccae* was administered to the contact (10^8 heat-killed bacilli/0.1ml intradermally into the left deltoid area). It was found that the patient was skin-test positive to common mycobacterial antigens, on testing after a period of two years from receiving the injection of *M. vaccae*. Re-testing results were as follows: left forearm: tuberculin=8mm, leprosin A=10mm; right forearm: scrofulin=5mm, vaccin=15mm.

3. This single leprosy contact demonstrates that immunotherapy with *M. vaccae* was able to confer recognition of common mycobacterial antigens (as judged for the positive skin responses to the 4 antigens) despite the subject had serological evidence of *T. cruzi* infection, an infection that has been associated to a lack of response to this kind of antigen.

EXAMPLE 4

This Example describes a study carried out on the influence of *M. vaccae* on a rat model of chronic myocarditis (induced by *Trypanosoma cruzi*). It is demonstrated that the amount of damage done to the heart of the rats in the rat model of chronic myocarditis was significantly reduced following immunotherapy with killed *M. vaccae*.

1. A study on the influence of *Mycobacterium vaccae* has been carried out in a rat model of chronic myocarditis induced by *Trypanosoma cruzi*. Male "I" rats derived from a cross between Rattus norvegicus and eIMM rats were used. 21 day old rats were inoculated with 10^6 living trypomastigotes of a Tulahuen strain of *Trypanosoma cruzi*.

2. 30 days post-infection, two groups of infected rats were given an intracutaneous injection of 0.1 ml of immunotherapeutic agent and 8 infected rats received an injection of saline. The immunotherapeutic agent was a suspension of autoclaved *Mycobacterium vaccae* strain NCTC 11659 in borate buffered saline. The first group of 10 rats received an injection of 10^7 *M. vaccae*, the second group of rats (8) an injection of 10^9 *M. vaccae*, and a group of 8 received an injection of saline.

3. 180 days post-infection, the heart was removed from ether-killed rats. Hearts were cut in the transverse

plane, fixed in neutral formalin and embedded in paraffin wax. Sections were cut for microscopy, 5μm thick, and stained with haematoxilin and eosin, or Masson's trichome stain. Four sections of each heart were studied carefully by a histopathologist experienced in pathology of the rat heart, blinded as to the experimental group from which the sections were derived. The degree of chronic myocarditis, defined by the presence of mononuclear cell infiltrate, myocyte degeneration of necrosis, and fibrosis was classified according to the extent of lesions and numbers of affected muscle fibres, into small, medium and large foci. Analysis of fibrosis was made by comparing prevalence of foci showing fibrosis between the experimental groups.

4. The results were as follows:

Low dose of *M. vaccae*	76% of foci were small
	12% of foci showed fibrosis
High dose of *M. vaccae*	57% of foci were small
	30% showed fibrosis
Placebo group	58% of foci were small
	52% showed fibrosis

The overall prevalence of fibrosis in rats receiving immunotherapy with 10^7 *M. vaccae* was significantly less than in the placebo group P=0.011. Therefore, the amount of myocarditis was shown to be less for the group of rats receiving the lower dose of *M. vaccae* than in the placebo group, with the group receiving the larger dose of *M.*

vaccae occupying an intermediate position.

5. The study demonstrates that immunotherapy with 10^7 killed *Mycobacterium vaccae* reduces the amount of damage done to hearts of the rat model of chronic myocarditis induced by *Trypanosoma cruzi*.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for the prophylaxis or treatment of vascular diseases or vascular complications which are immunologically mediated, which method comprises administering to the patient suffering from such a condition an effective amount of antigenic and/or immunoregulatory material derived from *Mycobacterium vaccae*.

2. A method according to claim 1, wherein the antigenic and/or immunoregulatory material derived from *M. vaccae* comprises dead cells of *M. vaccae*.

3. A method according to claim 2, wherein the cells of *M. vaccae* have been killed by autoclaving.

4. A method according to any one of the preceding claims, wherein the material derived from *M. vaccae* is derived from the strain as deposited at the National Collection of Type Cultures (NCTC) Central Public Health Laboratory, Colindale Avenue, London, NW9 5HT, United Kingdom on February 13th, 1984 under the number NCTC 11659.

5. A method according to any one of the preceding claims, wherein each dose contains antigenic and/or immunoregulatory material in an amount of from 10^7 to 10^{10} *M. vaccae* microorganisms.

6. A product comprising antigenic and/or immunoregulatory

material derived from *Mycobacterium vaccae* when used in the prophylaxis or treatment of vascular diseases or vascular complications which are immunologically mediated.

7. A product according to claim 6, wherein the material derived from *M. vaccae* is as defined in any one of claims 2 to 5.

8. A product according to claim 6 or claim 7 which further comprises a pharmaceutically acceptable carrier or diluent.

9. Use of antigenic and/or immunoregulatory material derived from *Mycobacterium vaccae* in the manufacture of a therapeutic agent for the prophylaxis or treatment of vascular diseases or vascular complications which are immunologically mediated.

10. Use according to claim 9 wherein the antigenic and/or immunoregulatory material derived from *M. vaccae* comprises dead cells of *M. vaccae*.

11. Use according to claim 10 wherein the cells of *M. vaccae* have been killed by autoclaving.

12. Use according to any one of claims 9 to 11 wherein the material derived from *M. vaccae* is derived from the strain as deposited at the National Collection of Type Cultures (NCTC) Central Public Health Laboratory,

Colindale Avenue, London NW9 5HT, United Kingdom on February 13th, 1984 under the number NCTC 11659.

13. Use according to any one of claims 9 to 12, wherein the therapeutic agent contains, per dose, antigenic and/or immunoregulatory material in an amount of from 10^7 to 10^{10} *M. vaccae* microorganisms.

Dated this 7th day of April 1999

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